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MicroCommentary

Cell wall growth during elongation and division: one ring to bind them?

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Summary

The role of the cell division protein FtsZ in bacterial cell wall (CW) synthesis is believed to be restricted to localizing proteins involved in the synthesis of the septal wall. In this issue of Molecular Microbiology, the groups of Christine Jacobs-Wagner and Waldemar Vollmer provide compelling evidence that in *Caulobacter crescentus*, FtsZ plays an additional role in CW synthesis in non-dividing cells. During elongation (cell growth) FtsZ is responsible for the incorporation of CW material in a zone at the midcell by recruiting MurG, a protein involved in peptidoglycan (PG) precursor synthesis. This resembles earlier findings of FtsZ mediated PG synthesis activity in *Escherichia coli*. A role of FtsZ in PG synthesis during elongation forces a rethink of the current model of CW synthesis in rod-shaped bacteria.

The shape of a bacterium is determined by its cell wall, but how the bacterium shapes its wall is not fully understood. The current view of cell shape generation in bacteria is that distinct modes of peptidoglycan (PG, the primary cell wall component) synthesis ultimately define shape. PG is a complex polymer, built up out of glycan chains that are interconnected via peptide crossbridges. Coccoid bacteria only synthesize PG at the plane of division (the septum), which bulges out after cell division leaving a round cell. Cells with a more complex shape, such as rod-shaped cells, exhibit an additional growth mode responsible for cell elongation (reviewed in Scheffers and Pinho, 2005). The two growth modes differ in orientation (perpendicular to the wall for division, parallel for elongation) and timing, as septal growth requires the formation of the FtsZ ring that triggers cell division. A classic study in *Escherichia coli* showed that some proteins involved in PG synthesis (so-called penicillin binding proteins, PBPs) are exclusively associated with either elongation or division (Spratt, 1975). This suggested that the two distinct modes of cell wall growth are catalysed by different sets of PBPs. Ever since, an important question has been how the two growth modes are co-ordinated in space and time. Division growth is ultimately co-ordinated by FtsZ. Evidence for a blueprint for elongation growth came from the identification of shape mutants that lack a clear association with cell wall synthesis. These mutations, located in the *mreBCD* genes, result in loss of rod-shape in *E. coli* and *Bacillus subtilis*. The discoveries that MreB is in fact the bacterial homologue of actin, that cells lacking MreB become spherical, that MreB forms helical structures underneath the membrane, and that MreB is absent from bacteria with a coccoid morphology all suggested that bacterial actin controls cell wall growth during elongation and is responsible for maintenance of cell width (Jones *et al*., 2001). This nicely complemented the role for the bacterial tubulin homologue FtsZ in division, and resulted in a model in which bacterial cell wall growth is ultimately co-ordinated by an internal cytoskeleton that provides a scaffold for the assembly of proteins involved in PG synthesis during elongation and division (Fig. 1A). Support for this model came from studies showing that some PBPs associated with elongation localize in patterns that are consistent with an underlying helical pattern in *E. coli, B. subtilis* and *Caulobacter crescentus* (see Scheffers and Pinho, 2005). A high–resolution technique to visualize nascent PG with fluorescent antibiotics revealed a helical pattern of PG synthesis along the lateral wall of *B. subtilis*, consistent with the pattern formed by MreB, although it is debated whether this localization is directly dependent on actin homologues (Daniel and Errington, 2003; Tiyanont *et al*., 2006).

In the current article Aaron *et al*. (2007) present compelling evidence that FtsZ is also involved in co-ordination of PG synthesis during elongation, which disagrees with the model described above. Aaron *et al*. investigated cell wall synthesis in *C. crescentus*, a Gram-negative bacterium that undergoes a developmental cycle.

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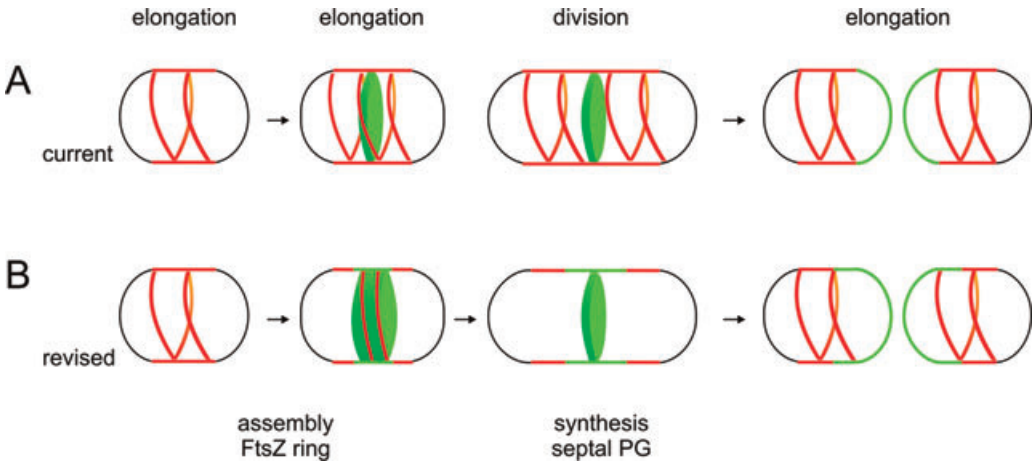

Fig. 1. A revised model for growth of rod-shaped bacteria.
A. In the current model, PG synthesis during elongation follows a helical or disperse pattern, controlled by the helical actin-like MreB filaments and/or MreC (red). FtsZ (green) mediates the assembly of the septal PG synthesis machinery.
B. In the revised model, PG synthesis during elongation is partially controlled by FtsZ. First, elongation is again mediated by helical PG insertion controlled by MreB, but after FtsZ localization to midcell, PG synthesis is increased at midcell and the MreB helices condense. Subsequently, septal PG is synthesized. New cell wall material derived from MreB- and FtsZ-controlled processes is indicated in red and green respectively. Note that in the revised model, a significant part of the sidewall is the result of FtsZ controlled elongation. A model for *C. crescentus* PG synthesis is presented by Aaron *et al.* (2007).

C. crescentus divides into two distinct types of cells, division-competent stalked cells and shorter, flagellated swarmer cells that cannot divide. Swarmer cells shed their flagellum and develop into stalked cells before being able to divide. *C. crescentus* cells are curved rods. Curvature is controlled by the intermediate filament homologue crescentin (Ausmees *et al.*, 2003), but because *C. crescentus* rod-shape is controlled by MreB (Figge *et al.*, 2004), it is thought that *C. crescentus* behaves like other rod-shaped bacteria, with MreB controlling PG growth during elongation and FtsZ controlling PG growth during division. Fluorescent antibiotics do not pass the outer membrane of Gram-negative cells, so to visualize PG synthesis Aaron *et al.* used the D-Cysteine (D-Cys) labelling technique pioneered by Miguel de Pedro (de Pedro *et al.*, 1997). D-Cys from the growth medium is uniformly incorporated into PG in exchange for the terminal residue on PG stempeptides that are subsequently detected by biotinylation and microscopy. This allows the study of PG fate, as the cells can be chased with medium without D-Cys for several generations after labelling. Thus, absence of label indicates turnover and insertion of new material whereas retention of label reveals zones of so-called 'inert' PG. To their surprise, the authors found that a large amount of PG synthesis in *C. crescentus* occurs at midcell. Labelled cells that were chased for one generation showed a large area of clearance around midcell. Midcell synthesis seemed to be responsible for cell elongation between the initiation of the stalked cell stage and cell division. Very little dilution of label was observed along sidewalls, as would be expected when elongation is solely the result of incorporation of material along the sidewalls. Aaron *et al.* conclude that an important part of cell wall growth during elongation is derived from localized PG synthesis at midcell. By applying the D-Cys labelling technique to FtsZ-depleted cells, the authors showed that localized midcell PG synthesis is controlled by FtsZ.

To study the link between FtsZ and cell wall synthesis further, the authors undertook careful microscopy of the localization of FtsZ, MreB and MurG. MurG is a membrane-associated cytoplasmic enzyme that catalyses the final step in the synthesis of the PG precursor Lipid II, which is required for PG synthesis irrespective of time and place. The choice for MurG rather than PBPs was an inspired one, as a clear correlation between the appearance of the FtsZ ring and an accumulation of MurG at midcell was found. MurG colocalizes with FtsZ just after formation of the FtsZ ring, well before the start of division (as judged by constriction). After division, the FtsZ ring dissolves and MurG delocalizes to a disperse membrane associated pattern. Similarly, MreB condenses from helices into a midcell ring in an FtsZ-dependent manner at the time of division, but this ring dissolves at an earlier stage during division than the MurG ring. Midcell accumulation of MurG depends on FtsZ but not on MreB or the presence of substrate, as shown by experiments in which FtsZ was depleted, MreB ring formation was inhibited or PG precursor synthesis was blocked. A careful analysis of FtsZ dynamics (see Movie S1 in *Supplementary material* of Aaron *et al.*, 2007) showed that the FtsZ ring is highly mobile prior to constriction, that it occupies a central region of the cell shortly after the swarmer to stalked cell

transition, and that the ring only becomes stationary just before constriction begins. Combined, these data lead to a new model of PG growth in *C. crescentus*. During division, PG synthesis is localized at midcell and is FtsZ-dependent. During elongation, two different growth modes exist: dispersed incorporation of PG in the absence of the FtsZ ring and localized insertion of PG in a zone at midcell co-ordinated by FtsZ (Fig. 1B). It is not unlikely that the dispersed incorporation is in fact helical and dependent on MreB and the MreB-associated membrane protein MreC. Both MreB and MreC are required for the helical localization of the elongation specific PBP2 in *C. crescentus* that, strikingly, relocalizes to midcell in the absence of MreB and MreC (Divakaruni *et al*., 2005; Dye *et al*., 2005). How much each mode contributes to cell elongation is unknown but a significant contribution is made by the FtsZ-dependent mode, as shown by the D-Cys labelling and the fact that stalked progeny cells elongate after the assembly of the Z ring. Finally, the authors note that stalk formation is the result of localized PG synthesis at the base of the stalk, but no accumulation of either MurG, MreB or FtsZ is observed at the base of the stalk. So either MurG-GFP does not accumulate to sufficient levels at the stalk base to allow localized detection, or stalk synthesis is controlled by another mechanism.

The most important contribution of this work is that it conclusively shows that FtsZ plays a role in PG synthesis during elongation. The question is whether this finding is specific for *C. crescentus* or whether there is evidence in the literature that FtsZ plays a similar role in other bacteria. The strongest evidence, as pointed out by Aaron *et al.* comes from *E. coli*. In *E. coli*, a burst of PG synthesis activity is observed at midcell. This activity is dependent on FtsZ but not on the activity of the cell division specific PBP3 (reviewed in Scheffers and Pinho, 2005). In fact, there is a significant time gap between formation of the FtsZ ring and the assembly of the septal PG synthesis apparatus (Aarsman *et al*., 2005). The increased PG synthesis activity during this time gap was interpreted as synthesis of preseptal PG, which is subsequently used to form the septum by the division specific PBPs. However, there is no evidence to exclude the possibility that this FtsZ-dependent PG synthesis is linked to elongation. In fact, elongation-specific PBP2 is present and active at the site of division without being stably associated with other cell division proteins (Den Blaauwen *et al*., 2003). Also, in the absence of FtsZ, shape defects in PBP mutants that are not linked to cell division are exacerbated (Varma and Young, 2004). In *Rhodobacter sphaeroides* several proteins involved in PG synthesis localize in a band at midcell, making it likely that midcell PG synthesis contributes significantly to growth (Slovak *et al*., 2006). In *B. subtilis*, labelling of nascent PG reveals high PG synthesis activity at the midcell in zones that appear wider than the actual septum, which might also be partially associated with elongation (Daniel and Errington, 2003; Tiyanont *et al*., 2006). Aaron *et al*. propose that the timing of the assembly of the FtsZ ring during the cell cycle controls the contribution of FtsZ-associated elongation growth.

The observation that FtsZ is not merely involved in PG synthesis during cell division but also during elongation prompts several new questions. First of all: how much do the FtsZ-dependent and –independent modes of synthesis contribute to elongation? A careful examination of the elongation rates of wild-type cells versus FtsZ depleted filaments could answer this question, although it is possible that delocalized MurG allows PG synthesis to proceed at a similar rate all over the filaments in the absence of FtsZ. Examination of FtsZ ring assembly and PG synthesis activity in several bacteria should establish whether, as Aaron *et al*. hypothesize, the length of the gap between ring formation and initiation of division controls the contribution of FtsZ mediated growth to elongation. Also, does the link between FtsZ and MurG localization result from a direct protein–protein interaction? After division, FtsZ forms a bright spot at the swarmer cell pole, whereas MurG becomes disperse, suggesting that MurG either interacts only with polymeric FtsZ or that an additional, FtsZ-associated protein is required.

Another important question raised by this work concerns the role of MreB in cell wall synthesis. The FtsZ-independent elongation growth, as stated above, could very well be dependent on MreB and MreC. MreB, MreC and PBP2 all localize in helical patterns, PBP2 depends on MreB/MreC for its correct localization and PBP2 and MreC interact (Divakaruni *et al*., 2005; Dye *et al*., 2005). However, MreB relocalizes to the FtsZ ring during division independently of MreC, suggesting that MreB might play a role in FtsZ-dependent PG synthesis during elongation and division. MurG localization was not dependent on MreB, but D-Cys labelling of cells producing the $MreB_{Q26P}$ variant that is defective in ring formation (Aaron *et al*., 2007) could reveal a function for MreB in PG-synthesis downstream of MurG function. MreB has been implicated in polarity and cell division in *C. crescentus* and other bacteria (reviewed in Carballido-López, 2006). The cell wall synthesized during division ultimately forms the new pole of the progeny cells. Polar PG is 'inert', i.e. not subject to degradation. Inert PG is linked to formation of branches in *E. coli* shape mutants (de Pedro *et al*., 2003). In *C. crescentus*, MreB depletion leads to the aberrant placement of stalks and formation of ectopic poles (Wagner *et al*., 2005), and in *B. subtilis* overexpression of the MreB homologue Mbl results in branching (Carballido-López, 2006). These findings suggest a role for MreB during cell division in the positioning of proteins required

for polar maturation of PG into inert PG, which needs further examination. Interestingly, Aaron *et al*. describe that the cell wall of the new pole is remodelled in the first cell cycle after division, because it changes from an oblate to a more pointed shape.

The finding of Aaron *et al*. that FtsZ plays a role in cell wall growth during elongation as well as division – if indeed true for all rod-shaped bacteria – requires us to take our models back to the drawing board. The simple and attractive model that states that elongation and division are directed by MreB and FtsZ, respectively (Fig. 1A) has to be replaced by a model that is more complex (Fig. 1B). It will be an exciting but tough task to work out how cell wall growth during elongation and division is ultimately controlled. Many PG synthesis proteins are redundant, which makes it hard to identify the specific contributions of each individual protein.

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